Plant Physiol. (1993) 102: 1185–1191

Acquisition of Desiccation Tolerance and Longevity in Seeds of *Arabidopsis thaliana*[1]

A Comparative Study Using Abscisic Acid-Insensitive *abi3* Mutants

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Two new abscisic acid (ABA)-insensitive mutants of *Arabidopsis thaliana* affected in the *abi3* locus are described. These new mutants are severely ABA insensitive. Like the earlier described *abi3-1* and the ABA-deficient and -insensitive double mutant *aba,abi3*, these new mutants vary in the extent of ABA-correlated physiological responses. Mutant seeds fail to degrade chlorophyll during maturation and show no dormancy, and desiccation tolerance and longevity are poorly developed. Carbohydrate accumulation as well as synthesis of LEA or RAB proteins are often suggested to be essential for acquisition of desiccation tolerance. In this work two points are demonstrated. (a) Accumulation of carbohydrates as such does not correlate with acquisition of desiccation tolerance or longevity. It is suggested that a low ratio of mono- to oligosaccharides rather than the absolute amount of carbohydrates controls seed longevity or stability to desiccation tolerance. (b) Synthesis of a few assorted proteins, which is responsive to ABA in the later part of seed maturation, is not correlated with desiccation tolerance or longevity.

ABA plays an important role in the development and maturation of seeds (Black, 1991). This hormone is essential for the induction of seed dormancy, as shown in experiments with ABA-deficient mutants (Karssen et al., 1983), and it is associated with the acquisition of desiccation tolerance during seed development (Koornneef et al., 1989). The ABA-deficient and -insensitive mutants of *Arabidopsis thaliana* enable the elucidation of crucial factors involved in desiccation tolerance. Three different loci for ABA insensitivity, designated *abi1*, *abi2*, and *abi3*, have been described (Koornneef et al., 1984). The *abi1* and *abi2* mutants suffer from disturbed water relations in the vegetative stage (i.e. wilting under water stress), whereas the *abi3* mutation specifically affects seed development (Koornneef et al., 1984). Seeds have reduced amounts of storage proteins and eicosenoic acid, the main fatty acid component of storage lipids (Finkelstein and Somerville, 1990). Seeds of ABA-deficient as well as ABA-insensitive mutants acquire desiccation tolerance, which may be due to leakiness of the mutations. However, the construction of the ABA-deficient and -insensitive double mutant *aba,abi3* resulted in plants that produce viable but desiccation-intolerant seeds. This desiccation-sensitive genotype allows a detailed analysis of desiccation tolerance in seeds (Koornneef et al., 1989; Meurs et al., 1992).

In several reports on desiccation tolerance, the main focus has been on carbohydrates and proteins (Kermode, 1990; Leopold, 1990; Skriver and Mundy, 1990). Carbohydrates may play an essential role in the acquisition of desiccation tolerance (Crowe et al., 1984; Leopold, 1990). One of the suggested functions of carbohydrates is membrane protection upon dehydration. Withdrawal of water molecules from the phospholipids can lead to membrane phase transitions at physiological temperatures (Crowe et al., 1989; Hoekstra et al., 1989). When water is available, these phase transitions coincide with membrane leakage and cell death. Hoekstra et al. (1991) have shown that carbohydrates can suppress the temperature of phospholipid phase transitions and prevent leakage of cellular solutes. In addition to the protection of phospholipids, carbohydrates are involved in the stabilization of proteins and retention of enzymic activity during dehydration (Carpenter and Crowe, 1988).

A second mechanism in which sugars are probably involved is in the formation of a glass during dehydration (Burke, 1986). A glass is a liquid of high viscosity, such that it stops all chemical reactions requiring molecular diffusion and might function in conserving tissue structures during dehydration. Comparing desiccation-tolerant and -intolerant tissues, Bruni and Leopold (1991) found that all tolerant tissues had formed a glassy state, whereas the intolerant tissues had not. In vitro studies have shown that the relative amounts of different sugars can influence the stability of the glassy state at physiological temperatures (Koster, 1991).

Besides carbohydrates, the synthesis of specific proteins might also be involved in the acquisition of desiccation tolerance. From studies of the *aba,abi3* double mutant it is known that accumulation of storage proteins is inhibited

[1] This research was financially supported by the Bridge program of the European Community (J.J.J.O. and K.M.L.-K.).

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Abbreviation: dap, days after pollination.

(Koornneef et al., 1989; Meurs et al., 1992). Instead of accumulating maturation-related proteins, *aba,abi3* seeds synthesize germination-related proteins in the later stages of development (Meurs et al., 1992). The absence of maturation-related proteins in *aba,abi3* seeds agrees with the general view that ABA and/or dehydration stimulate the synthesis of proteins that are involved in the protection from desiccation damage (Skriver and Mundy, 1990; Black, 1991).

In this study, we introduce two new *abi3* mutant alleles that allow normal plant growth but keep seeds green until maturity. The mutants are characterized by a reduced desiccation tolerance and/or longevity. The seed phenotypes of these new ABA-insensitive mutants strongly resemble the recently described *abi3-3* mutant seeds (Nambara et al., 1992) and the *aba,abi3* double-mutant seeds (Koornneef et al., 1989). The *abi3* gene has recently been cloned (Giraudat et al., 1992) and encodes a protein with distinct regions of homology to the maize *vp1* product (McCarthy et al., 1991), which is suggested to be a transcriptional activator and may potentiate ABA responses in the maize embryo. We studied the effect of ABA insensitivity on dormancy development, acquisition of desiccation tolerance, and longevity in seeds with three different *abi3* alleles and in seeds of the *aba,abi3* double mutant and wild type. The use of mutants that are gradually different in ABA insensitivity facilitates the elucidation of the role of carbohydrates and proteins in the acquisition of desiccation tolerance and longevity.

MATERIALS AND METHODS

Plant Material

All genotypes used have been derived from *Arabidopsis thaliana* ecotype Landsberg *erecta* (wild type). The *abi3-1* mutant (isolation number CIV) and the *aba-a,abi3-1* double mutant (isolation numbers A26 and CIV, respectively) have been described by Koornneef et al. (1982, 1984, 1989). The mutant line SM1 was generated in *A. thaliana* Landsberg *erecta abi3-1* seeds by applying 15 mM ethylmethanesulfonate for 24 h. The SM1 mutant was isolated as a dark green M_2 seed, which germinated immediately after harvest. The mutant line 10286 was kindly provided by Dr. L. Conway (University of Pennsylvania, Philadelphia) as an M_3 line, segregating for green seeds. It was generated by treating *A. thaliana* Landsberg *erecta* seeds with 11 mM diepoxybutanol for 4 h. To test allelism, crosses were made between 10286 and SM1 and between 10286 and CIV. The F_1 seeds were sown in Petri dishes containing filter paper saturated with 10 μM ABA and incubated at 25°C, with a 16-h light period. Germination was scored after 7 d. For mapping, crosses were made between SM1 and lines containing the visible markers *hy2* and *gl1*. F_2 populations derived from these crosses were scored for plants with brown, green, and a mixture of brown and green seeds together with the marker phenotypes.

For plant culture, seeds were sown in Petri dishes on water-saturated filter paper. The Petri dishes were placed in an incubator at 25°C under continuous light (8 W/m^2). After 2 to 4 d, germinated seeds were transferred into soil and cultivated in a greenhouse (18–22°C) with additional light (Philips TLD 58W/84, 16-h light period).

Seed Collection

Mature seeds were harvested from dehydrated siliquae. Staging of the developing seeds was performed by tagging individual flowers on the day of anthesis. For the analyses of dormancy, desiccation tolerance, and carbohydrate content, collection of young seeds during development was carried out in a glove box under 100% RH to avoid possible changes due to moisture loss. Immature seeds were collected by opening unripe siliquae using a needle and forceps. Collected seeds were immediately placed on water-saturated filter paper or dry filter paper or immersed in 80% methanol for the analysis of dormancy, desiccation tolerance, and carbohydrates, respectively.

Germination Assays

To determine the sensitivity of germination to ABA, 40 to 80 mature seeds were sown in triplicate in Petri dishes containing filter paper soaked with a range of ABA concentrations. The Petri dishes were stored for 4 d at 4°C and subsequently incubated in a growth chamber (25°C, 16-h light period). Germination was scored 7 d after the start of incubation at 25°C. To determine dormancy in developing seeds, the contents of at least two siliquae (40–60 seeds) were placed in triplicate in Petri dishes containing filter paper soaked with water. Germination was scored after a 14-d incubation at 25°C in continuous light. To determine desiccation tolerance in developing seeds, the contents of at least two siliquae (40–60 seeds) were placed in triplicate in open Petri dishes containing dry filter paper and the Petri dishes were placed in an incubator (30% RH, 25°C). After 2 d, the filter paper was soaked with 100 μM GA_{4+7}. Germination was scored after 14 d of incubation at 25°C in continuous light.

Carbohydrate Analysis

Approximately 50 seeds were homogenized in 0.5 mL of 80% methanol with 25 μg of melezitose as the internal standard. The homogenate was boiled for 15 min at 75°C and the methanol was evaporated under vacuum at 20°C. The dried pellet was taken up in water, and the suspension was centrifuged in an Eppendorf centrifuge (5 min, maximum speed). The supernatant was directly injected in a Dionex HPLC system (Dionex Corp., Sunnyvale, CA) equipped with a Carbopac PA100 column and a pulse-amperometric detection system. Samples were eluted isocratically with 0.1 N NaOH. Carbohydrates were identified by comparing chromatographs with standards under two different elution protocols.

Protein Extraction

For protein extraction, dry mature seeds or intact immature siliquae were frozen in liquid nitrogen. Either approximately 3 mg of dry seeds or six siliquae were used for one extraction and homogenized in 100 μL of sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.025% bromphenol blue). Proteins were separated on a 12% SDS polyacrylamide gel and electroblotted from the gel to a nitrocellulose membrane. The membranes were probed with

rabbit antiserum (1:1000 diluted) raised against ABA-induced proteins from *Craterostigma plantagineum* (Schneider et al., 1993) or aldose reductase from barley (Bartels et al., 1991), and further incubated with anti-rabbit immunoglobulin G peroxidase conjugate (Sigma). The protein antibody complex was detected using the chemiluminescence (ECL) western blotting detection system from Amersham (UK).

RESULTS

Genetic Analysis of the SM1 and 10286 Mutant

To test allelism, crosses were made between 10286 and SM1 and between 10286 and *abi3-1*. The F_1 seeds from these crosses germinated within 2 to 3 d and, in addition, were able to germinate in the presence of 10 μM ABA. Lack of dormancy and this insensitivity to ABA demonstrate allelism. Linkage analysis using F_2 data from crosses between SM1 and marker lines revealed linkage between the SM1 mutation and *hy2* and *gl1* with estimates of recombination percentages of 31.3 ± 2.3 and 8.6 ± 1.1 centiMorgan, respectively. These linkage data are in accordance with the previously published map position of *abi3* (Koornneef and Hanhart, 1984). These data together indicate that the mutant lines SM1 and 10286 have mutations in the *ABI3* locus. We designate these new alleles *abi3-4* (SM1) and *abi3-5* (10286).

Germination Tests

For a preliminary physiological characterization of the various *abi3* mutants in comparison with wild type and the *aba-1,abi3-1* double-mutant seeds, we tested germination behavior under different conditions and at different stages of development. Figure 1 shows the sensitivity of mature seeds to the ABA-induced inhibition of germination. It is clear that germination of wild-type seeds is fully inhibited at 10 μM ABA, whereas similar inhibition of *abi3-1* seed germination occurs at 100 μM ABA. In contrast, germination of seeds from both new *abi3* mutations (*abi3-4* and *abi3-5*) could be only slightly inhibited by 1 mM ABA. This strong insensitivity to ABA of the *abi3-4* and *abi3-5* mutant seeds correlates with the more severe phenotype of the *abi3-4* and *abi3-5* alleles as compared with the *abi3-1*. The alleles strongly resemble the green seeds of the ABA-insensitive and -deficient double mutant *aba-1,abi3-1*, in which ABA action is strongly reduced, if present at all (Koornneef et al., 1989), and the extreme *abi3-3* allele described by Nambara et al. (1992).

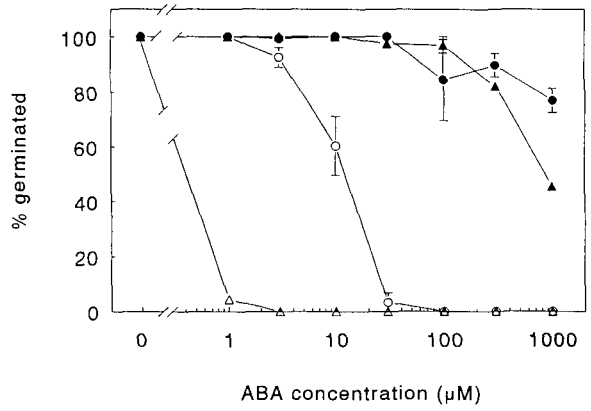

Figure 1. Germination capacity in the presence of ABA. Freshly harvested seeds from mature siliquae of wild type (Δ) and the mutants *abi3-1* (O), *abi3-4* (▲), and *abi3-5* (●) were tested for their ability to germinate in different ABA concentrations. Data are averages ± SD of triplicate determinations.

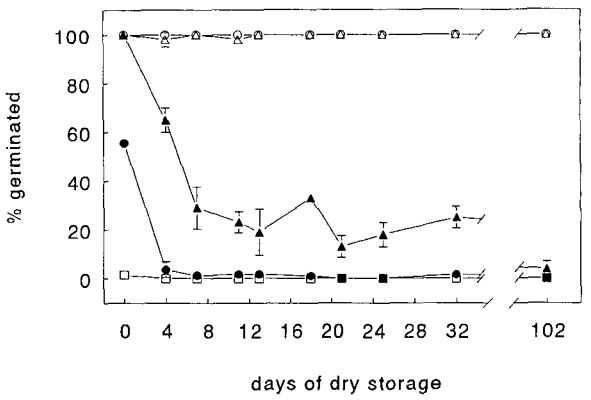

Figure 2. Effect of the duration of dry storage (30% RH, 25°C) on germination capacity of seeds from mature siliquae. Seeds were wild type (Δ), *abi3-1* (O), *abi3-4* (▲), *abi3-5* (●), and *aba-1,abi3-1* (□). Data are averages ± SD of triplicate determinations.

We further tested these seeds for survival during dry storage. Seeds from mature dry siliquae were stored at 25°C and 30% RH. After different storage periods, seeds were tested for germination in 100 μM GA_{4+7} (Fig. 2). Wild-type and *abi3-1* seeds can withstand long periods of dry storage. The double mutant *aba-1,abi3-1* does not survive desiccation at all, whereas the *abi3-4* and *abi3-5* seeds are intermediate in this respect.

In Figure 3, A and B, germination capacities during seed development of freshly harvested and dried mutant seeds are shown and compared with those of wild-type seeds. Seeds of all genotypes except the double mutant *aba-1,abi3-1* acquire desiccation tolerance, which seems to be transient in the case of *abi3-5* (Fig. 3B). The loss of desiccation tolerance in *abi3-5* seeds 16 dap might be caused by precocious germination due to the absence of dormancy (Fig. 3A). Freshly harvested wild-type seeds do not germinate from 16 dap onward, because they reach full dormancy at this stage. Of the other genotypes, only *abi3-1* seeds develop some dormancy (Fig. 3A). The degree of dormancy of the seeds carrying *abi3* alleles correlates with their ABA sensitivity (Fig. 1). The reduced germination of *aba-1,abi3-1* double-mutant seeds at 20 dap is caused by dehydration of seeds in the siliquae prior to the germination test.

Carbohydrates

To investigate the role of soluble carbohydrates in the acquisition of desiccation tolerance, we analyzed the carbohydrate composition during seed development of the five genotypes (Fig. 4, A-E). Due to the low seed weight, ranging from 14 to 20 μg for dry mature seed, an accurate determi-

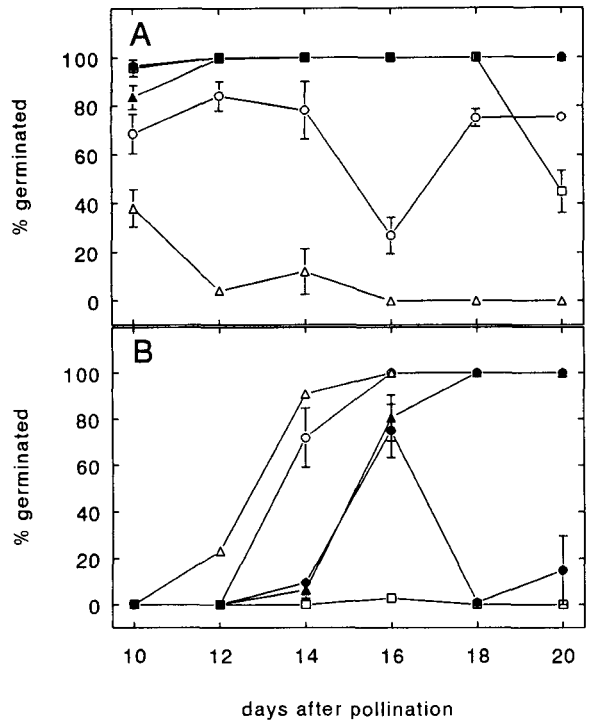

Figure 3. Germination capacity of developing *Arabidopsis* seeds. Freshly harvested developing seeds (A) were germinated on water. Dried seeds (B) (48 h, 24°C, 30% RH) were germinated on 100 μM GA_{4+7}. Seeds were wild type (Δ), *abi3-1* (O), *abi3-4* (▲), *abi3-5* (●), and *aba-1,abi3-1* (□). Data are averages ± SD of triplicate determinations.

nation of dry weights during the course of seed development is hardly possible. Thus, carbohydrate content during seed development is expressed in μg/100 seeds. Figure 4, A and B, reveal that the amount of Glc and Fru are more or less equal in all genotypes until 16 dap. From 16 dap onward, the amount of Glc and Fru strongly increases in *aba-1,abi3-1* double-mutant seeds, whereas all other genotypes show a small decrease in monosaccharide content. Raffinose and stachyose, although present in minor quantities, increase in amount in the later stages of maturation in wild-type and *abi3-1* seeds (Fig. 4, C and D). In the *abi3-4*, *abi3-5*, and *aba-1,abi3-1* double-mutant seeds, the amounts of raffinose and stachyose are below the detection limit. Compared with mature wild-type seeds, the different mature mutant seeds have three to five times more Suc (Fig. 4E).

In Table I, the carbohydrate data from Figure 4 are summarized and the ratio of mono- to oligosaccharides in mature seeds has been calculated. These data suggest a correlation between a low mono-/oligosaccharide ratio and desiccation tolerance. However, this correlation was not found for immature, desiccation-tolerant developing seeds. Nevertheless, a correlation between the mono-/oligosaccharide ratio and longevity cannot be excluded.

Proteins

We tested whether proteins present in *Arabidopsis* seeds cross-reacted with antibodies raised against ABA- or dehydration-induced proteins of the desiccation-tolerant resurrection plant *Craterostigma plantagineum* and barley embryos (Piatkowski et al., 1990; Bartels et al., 1991). Antibodies raised against pcC6–19 and pcC27–45 cDNA-derived polypeptides overexpressed in *Escherichia coli* were used. The protein encoded by pcC6–19 shows homology with proteins expressed during late embryogenesis in seeds of higher plants. Proteins encoded by pcC27–45 are suggested to be characteristic of *Craterostigma* (Piatkowski et al., 1990). The cDNA clone encoding pG22–69 has been isolated from desiccation-tolerant barley embryos and shows structural homology to NADPH-dependent aldose reductase synthesized in mammals (Bartels et al., 1991). Figure 5 shows immuno-western blots of *Arabidopsis* protein extracts from seeds of different developmental stages incubated with the pcC6–19 antibody. It illustrates that in the later stage of seed development, all genotypes, except the *aba-1,abi3-1* double mutant, synthesize at least one protein that is immunologically related with pcC6–19. With the antibody against the *Craterostigma*-specific protein pcC27–45, no cross-reaction was found (not shown). In the wild-type and *abi3-1* seeds, some cross-reaction is found with antibodies directed against the polypeptide encoded by pG22–69 from barley (not shown).

DISCUSSION

In this study, we introduce two new ABA-insensitive *Arabidopsis* mutants that are affected in the *ABI3* locus, as can be concluded from the complementation tests and map position. In contrast to the *abi1* and *abi2* mutants, the *abi3* mutations specifically affect seed development (Koornneef et al., 1984; Finkelstein and Somerville, 1990; Nambara et al., 1992). ABA is generally known to play a crucial role in seed maturation and acquisition of desiccation tolerance (Koornneef et al., 1989; Black, 1991). Consequently, it can be expected that a comparative study using these mutants can contribute to the understanding of seed maturation and desiccation tolerance.

The capacity of a seed to germinate after being stored in the dry state is often used as a measure of desiccation tolerance. However, storage time and residual moisture content are generally not clearly defined. For practical reasons, we define desiccation tolerance as the capacity of a seed to grow into normal seedlings after a 48-h desiccation period at 25°C and 30% RH. Under these conditions, the water content of the seeds is reduced to approximately 5% of the dry weight. Survival of dry storage for longer periods is covered by the general term longevity.

Comparison of Figure 1, in which sensitivity of germination to ABA is tested, with survival of dry storage as shown in Figure 2 suggests a positive correlation between ABA sensitivity and longevity. In the course of seed development, wild-type seeds are the first to acquire desiccation tolerance, followed by *abi3-1*, *abi3-4*, and *abi3-5* seeds (Fig. 3B). The *aba-1,abi3-1* double-mutant and the *abi3-5* seeds acquire a transient desiccation tolerance around 16 dap (see also figure 2B in Koornneef et al., 1989). The immediate loss of desiccation tolerance in these two genotypes is possibly due to precocious germination in the siliquae caused by the high RH

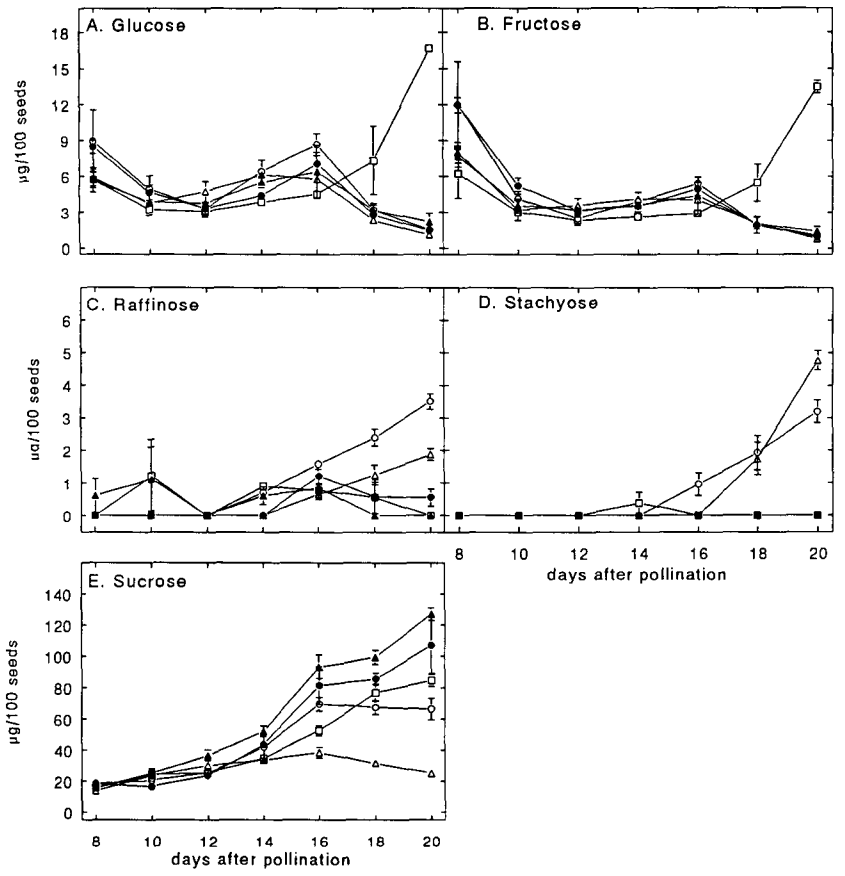

Figure 4. Accumulation of carbohydrates during seed development, expressed in µg/100 seeds. A, Glc; B, Fru; C, raffinose; D, stachyose; and E, Suc; note the scale differences. Seeds were wild type (Δ), *abi3-1* (○), *abi3-4* (▲), *abi3-5* (●), and *aba-1,abi3-1* (□). Data are averages ± SD of triplicate determinations.

at which plants are grown and the lack of dormancy in these seeds.

The extent of germination of freshly harvested seeds (Fig. 3A) in the later stages of development correlates with ABA insensitivity (Fig. 1). The severe ABA insensitivity also correlates with the absence of Chl breakdown during seed maturation and, according to Nambara et al. (1992), the absence of 12S and 2S proteins. Dormancy develops in wild-type and, to a small extent, in *abi3-1* seeds, whereas it is absent from the new *abi3-4* and *abi3-5* mutant seeds and the *aba-1,abi3-1* double mutant (Koornneef et al., 1989). These results confirm the earlier established role of ABA in seed development (Karssen et al., 1983; Koornneef et al., 1989). The data further support the essential role of the ABI3 protein as a regulator of seed maturation, which possibly acts in combination with ABA or an ABA-induced protein. The extreme insensitivity to ABA of the *abi3-4* and *abi3-5* alleles further indicates that the inhibitory effect of an excess of exogenous ABA is mediated by this ABI3 protein. The observation that *vp1* mutants are only slightly resistant to ABA (Robichaud et al., 1980) indicates that in this respect the *abi3* mutants differ from *vp1* mutants. In addition, no obvious effects on anthocyanin content are observed in the *abi3* mutants; anthocyanins do not accumulate in seeds of the

Table I. *Relative amounts of monosaccharides (Glc + Fru), disaccharide (Suc), and oligosaccharides (raffinose + stachyose) present in 20-dap (mature) seeds*

Values are calculated from the data in Figure 4.

Genotype	Relative Amounts of Carbohydrates			Ratio of Mono- to Oligosaccharide	Total Sugar Content
	Monosaccharide	Disaccharide	Oligosaccharide		
		%			*ng/mg dry weight*
Wild type	11.1	77.9	10.9	1.02	55.4
abi3-1	6.3	88.8	4.9	1.28	135.1
abi3-4	4.3	95.4	0.3	15.19	165.5
abi3-5	5.1	94.7	0.2	21.66	209.4
aba-1,abi3-1	40.2	59.6	0.1	354.12	296.6

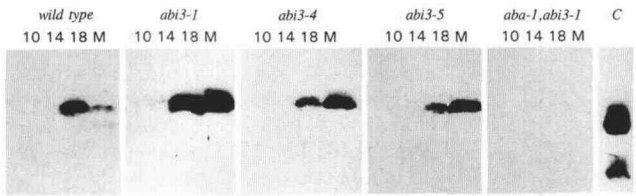

Figure 5. Western blot analysis. Protein extracts were prepared from of 10-, 14-, and 18-dap and M (mature) intact siliquae and incubated with pcC6–19 antiserum. The control lane (C) contains a crude protein extract from dried *Craterostigma leaves*. Note that band intensity is not a quantitative measure of protein amount.

maize *vp1* mutants (Hattori et al., 1992). However, especially in their effects on seed-specific and ABA-affected characters, the *abi3* and *vp1* mutants are similar, which together with the resemblance of the DNA sequence (Giraudat et al., 1992) suggests overlapping but probably not completely identical functions of these genes.

Many organisms in the dehydrated stage contain large amounts of soluble carbohydrates. Desiccation tolerance in seeds and pollen is associated with the accumulation of Suc and oligosaccharides like raffinose and stachyose (Amuti and Pollard, 1977; Hoekstra et al., 1989; Leopold, 1990). With the isolation of the *abi3–4* and *abi3–5* mutants, we obtained a series of *Arabidopsis* mutant seeds that differ in the acquisition of desiccation tolerance and longevity (Figs. 2 and 3B). With the use of these seeds, we examined whether desiccation tolerance correlates with accumulation of certain soluble carbohydrates. Only in the *aba-1,abi3–1* double mutant do the amounts of Glc and Fru strongly deviate from those found in wild-type seeds (Fig. 4, A and B). Only raffinose and stachyose accumulate in the desiccation-tolerant wild-type and *abi3–1* seeds, whereas this accumulation is minimal or not detected in the other genotypes (Fig. 4, C and D). However, accumulation of raffinose and stachyose is not temporally linked with acquisition of desiccation tolerance (compare Figs. 4, C and D, and Fig. 3B). The most desiccation-tolerant seeds, wild type and *abi3–1*, have the lowest amounts of Suc (Fig. 4E). Moreover, as illustrated in Table I, the genotypes that have the highest desiccation tolerance (Fig. 3B) accumulate the lowest total amounts of soluble sugars in the later part of seed development. The huge accumulation of carbohydrates in desiccation-intolerant tissues, together with the temporal separation of oligosaccharide accumulation and development of desiccation tolerance, leads us to the conclusion that accumulation of carbohydrates as such is not sufficient for acquisition of desiccation tolerance in *Arabidopsis* seeds.

Table I and Figure 2 suggest a correlation between a low mono-/oligosaccharide ratio and longevity. Stable glass formation, which is suggested to be essential for acquisition of desiccation tolerance (Bruni and Leopold, 1991), depends on the mono-/oligosaccharide ratio (Koster, 1991). Consequently, it is suggested that the declining longevity of the mutant seed of *abi3–4* and *abi3–5* could be caused by instable glass formation, eventually expressed as desiccation intolerance in *aba-1,abi3–1* double-mutant seeds.

Besides the direct protective aspects of carbohydrates, accumulation of carbohydrates will contribute to the osmotic potential of the seeds. It has been shown that not only ABA but also osmotic effects are important for normal seed development, acquisition of desiccation tolerance, and prevention of precocious germination (Finkelstein and Crouch 1986, 1987; Xu et al., 1990). The *aba-1,abi3–1* double-mutant seeds, which strongly accumulate carbohydrates, germinate precociously and synthesize germination-related proteins instead of maturation-related proteins in the later stages of seed development (Meurs et al., 1992). Consequently, the accumulation of carbohydrates in *abi3–4* and *abi3–5* seeds might correlate with precocious germination together with a similar change in protein pattern. However, our results indicate that seeds of all genotypes except the *aba-1,abi3–1* double mutant synthesize a maturation-related protein showing homology to the ABA- and desiccation-inducible protein encoded by cDNA clone pcC6–19, which was isolated from *Craterostigma plantagineum* (Fig. 5). Thus, it is not likely that in the *abi3–4* and *abi3–5* mutant seeds a changed protein pattern concomitant with precocious germination is the cause of a transient desiccation tolerance. Rather, the presence of this protein in all genotypes except in the double mutant suggests a correlation with desiccation tolerance. However, in 14-dap seeds, no detectable cross-reaction was found, whereas wild-type and *abi3–1* seeds have acquired nearly full desiccation tolerance at this stage of development. Thus, the expression of this protein is not temporally related to the acquisition of desiccation tolerance or longevity.

We also found cross-reaction with an antibody against pG22–69 in wild-type and *abi3–1* seeds (not shown). This indicates the presence of a protein in *Arabidopsis* seeds with homology to a functional aldose reductase in barley embryos (Bartels et al., 1991). Aldose reductase is known to be involved in the salt stress-induced conversion of Glc to sorbitol in mammalian cells and functions in osmoregulation (Perez et al., 1989). However, the presence of sorbitol has not been established in *Arabidopsis* seeds.

Our results show that the presence of endogenous ABA and the absence or negligible amounts of the ABI3 gene product is sufficient to induce desiccation tolerance as found in *abi3–4* and *abi3–5* seeds. Apparently, the endogenous ABA is not sufficient to extend longevity. The different patterns of carbohydrate accumulation in the ABA mutants are indicative of a role of ABA in the regulation of carbohydrate metabolism. The presence of a protein with homology to aldose reductase in the wild-type and *abi3–1* seeds, which are both desiccation tolerant and have an extended longevity, supports this idea. Accumulation of carbohydrates as such does not seem to be a prerequisite for desiccation tolerance. However, the composition of carbohydrates may influence seed longevity.

ACKNOWLEDGMENTS

We thank Dr. L. Conway for making available mutant line 10286, Dr. K. Schneider for pcC6–19 antiserum, Casper Pillen for technical assistance, and Folkert Hoekstra and Renske van der Veen for critical reading of the manuscript.

Received January 13, 1993; accepted April 16, 1993.

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